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Identification of a Highly Cross-Reactive Outer Surface Protein B Epitope among Diverse Geographic Isolates of *Borrelia* spp. Causing Lyme Disease

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The outer surface lipoprotein B (OspB) of *Borrelia burgdorferi* is a major component of the borrelial protein profile and has been shown to be highly immunogenic in experimentally immunized and infected mammals. However, the *ospB* loci of different strains show considerable heterology at the nucleic acid sequence level, and the progeny of a clonal strain of *B. burgdorferi* exhibited OspB polymorphisms with respect to apparent molecular weights and reactivities with monoclonal antibodies. These data suggest that OspB is not a good candidate for vaccination or diagnostic purposes. The present study describes a monoclonal antibody, designated 84C, directed against a very highly conserved domain of the OspB lipoprotein. Western immunoblot analysis with 84C demonstrated reactivity in 84.2% of human, tick, and other vertebrate isolate strains examined from widely diverse geographic regions, including strains of *B. burgdorferi* sensu stricto and two closely related species, *B. garinii* and *B. afzelii*. The 84C-binding region was delimited to a highly conserved 11-amino-acid region in the carboxyl terminus of OspB as demonstrated by (i) DNA sequence analysis of wild-type and 84C-resistant mutant *ospB* alleles and (ii) deletion mutagenesis of a recombinant *ospB* gene in *Escherichia coli*. Finally, the 84C epitope was demonstrated to be exposed on the borrelial surface in situ as (i) the monoclonal antibody 84C was able to agglutinate borrelias in culture and (ii) 84C-resistant escape variants were isolated. These data suggest that the potential value of OspB as a vaccine candidate or diagnostic tool be examined more closely, in the context of the 84C-reactive domain.

Lyme disease is a tick-borne spirochetosis which has reached high levels in some regions of the United States and is also a genuine health problem in other regions of the world, notably in northern Europe and Asia. The bacterial species responsible for the disease are members of the genus *Borrelia* and were formerly clustered in the species *B. burgdorferi* sensu lato (35). Recently, multiple independent laboratories have presented several lines of evidence splitting this single species into three or more species (1, 5, 14, 41, 45, 59). These include *B. garinii*, associated with the extradermally associated neurologic and cardiac syndromes of Lyme disease in Europe, and the recently described *B. afzelii* (17), formerly known as the VS461 group, which includes those borrelias associated with the cutaneous syndrome of erythema migrans or acrodermatitis chronica atrophicans among European patients (3, 14, 58). Currently, virtually all North American isolates have fallen into *B. burgdorferi* sensu stricto, regardless of the disease course in infection (3, 14, 59). For the purpose of simplicity, unless otherwise specified, the term Lyme disease borrelia will be used here to refer to a member of any of these three species.

Most of the Lyme disease borrelias express two major outer surface lipoproteins, known as OspA and OspB. The genes encoding these lipoproteins are observed to occur in an operonic arrangement (*ospAB*) on linear plasmids of ~50 kbp (36). The structural genes encoding these lipoproteins have been cloned and expressed in *Escherichia coli* (33). The lipoproteinaceous nature of these antigens was proposed in 1989 (11) and confirmed in 1990 by Brandt et al. (15). Many European isolates of Lyme disease borrelias have been reported to lack expression of OspA and/or OspB while exhibiting another immunologically heterogeneous, major outer surface protein of ~20 kDa, designated OspC and previously known as pC (60). The gene encoding OspC from several different borrelial strains has been cloned in *E. coli* (60) and is proposed to encode a lipoprotein (31). In *B. burgdorferi* B31, the *ospC* gene has been localized to a 26- or 27-kbp circular plasmid (42, 49).

Comparison of the similarities of OspA and OspB from the *B. burgdorferi* type strain B31 at the amino acid level indicates that the two genes are 53% identical to each other, suggesting a common evolutionary origin involving a recent duplication event (11). When the nucleic acid sequences of the *ospB* loci from three divergent borrelial strains were compared, 79% sequence identity was detected over their lengths, with most of the degeneracies observed occurring in the wobble position of each codon (36). At the amino acid level, better conservation was observed for the OspB amino termini as compared with the central and carboxy-terminal portions of the proteins (36). Additionally, in a subsequent report, while the OspB protein was still expressed by borrelias, variations were observed in the apparent molecular weights and reactivity with monoclonal antibodies (MAbs) of OspBs among clonal populations of *B. burgdorferi* HB19 (16). Additionally, Rosa et al. have described chimeric *ospAB* hybrid genes present in uncloned populations of

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several strains of Lyme disease borrelias (46). These data suggest a relatively high degree of instability for this lipoprotein, at least at the level of antigenicity. Another report indicated that the OspB lipoprotein showed a decrease in expression related to the number of passages in laboratory media (51). This drop in OspB expression may be related to nonsense point mutations in the *ospB* gene which terminate translation, as have been reported by several laboratories (30, 47). Data such as these have shifted emphasis on the utility of this major surface lipoprotein in diagnostics or vaccine potential to other "more faithfully" conserved or expressed borrelial antigens.

A number of studies have examined the degree of antigenic conservation of OspA and OspB among various isolates of Lyme disease borrelias, with a review of the data by Wilske et al. (61). Polyclonal antisera against the OspB of one European isolate (PAB) failed to react with two North American patient isolates (strains 272 and 297) and five other European cerebrospinal fluid isolates (PKa, PBr, PFei, PBi, and W12). Similarly, an anti-OspB antiserum generated against the North American tick isolate type strain B31 was also nonreactive for European strains with one exception, a German cerebrospinal fluid isolate (PKa [61]). Prior to this review, Barbour et al. reported in 1984 that only 6 and 11 of 14 strains tested reacted with the anti-OspB MAbs H6831 and H5TS, respectively (9). A survey of a larger set of strains in 1986 led to a report that only 16 of 45 strains (25 North American and 20 European) were reactive with H6831, while 44 of the 45 reacted with the anti-OspA MAb H5332 (8). These data underscore the large amount of heterogeneity that is observed among the OspA and OspB of the Lyme disease borrelias.

With regard to the functionality of the Osps, OspA has been tested as a vaccine candidate in mouse model systems and has been shown to confer both passive (28, 50) and active, acquired (28) protection from infection in a strain-specific manner. OspB has also been shown to confer some degree of acquired immunity to disease in a mouse model system (29, 30), suggesting the potential importance of this lipoprotein in pathogenesis. We have proposed potential roles for OspA in the attachment of borrelias to human endothelial cells and for OspB in the penetration of these cells as assessed in vitro (21, 47). However, the conclusive function(s) of any of the Osp proteins in the borrelia is undefined at the present time.

With these data in mind, we proposed to generate a pool of MAbs directed against the North American strain HB19, a human blood isolate (54), in an attempt to define surface antigens that played a role in attachment to and penetration of human endothelial cells in culture. The results of that study demonstrated one anti-OspA MAb, 9B3D, whose Fab fragments were able to inhibit the attachment of borrelias to endothelial cells, yet had no adverse effect on penetration of host cell monolayers, while seven other anti-OspA MAbs had no effect on borrelial attachment or penetration (21). That study also generated 14 other MAbs directed against three more borrelial antigens, including 8 against OspB, all of which had no effect on borrelial attachment or penetration of human endothelial cells. However, four of the MAbs, 9B1C and 9B2B (anti-OspA), BP1E10 (anti-19 kDa), and 84C (anti-OspB), demonstrated reactivity against an initial screen of 10 borrelial strains, including strains from Russia and Europe (20). One of these four, 84C, was characterized further in the present study and was observed to react with a larger percentage of strains tested than any other anti-OspB MAb reported to date. We desired to characterize the region of the OspB molecule that was recognized by 84C, and we determined by a two-pronged approach that 84C bound to a 43-amino-acid region in the carboxy terminus of the OspB lipoprotein. We propose that since the majority of strains which we have tested possess this antigenic structure, it may have some importance in the physiology of the Lyme disease borrelias or in the pathogenesis of Lyme disease. Therefore, we also propose that OspB merits additional study as a potential vaccine candidate or diagnostic tool in the context of the broadly conserved 84C epitope.

(Portions of these data were presented at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992.)

MATERIALS AND METHODS

Bacterial strains and media. *Borrelia* sp. strains used in this study are listed in Table 1. Borrelias were propagated at 34°C in BSK II liquid media (4) supplemented with 6% normal, unheated rabbit serum (GIBCO Laboratories, Grand Island, N.Y.). Borrelias grown in BSK II media fewer than or equal to 10 passages from the initial isolate are considered to be low passage, while those with greater than 10 passages are considered to be high passage in our laboratory. Washed, whole-cell extract (WCE) samples for Western immunoblot analysis were prepared by centrifugation of 5 to 10 ml of a late-exponential-phase borrelial culture ($\sim 0.5 \times 10^8$ to 1×10^8 borrelias per ml) at $12,000 \times g$ for 20 min at room temperature (RT). Pelleted borrelias were washed three times at RT in 1 ml of phosphate-buffered saline containing 5 mM $MgCl_2$ (pH 7.35; PBSM). Protein concentrations were estimated with a modified Bradford assay with bovine serum albumin as a standard (BioRad Laboratories, Richmond, Calif.).

The *E. coli* strains DH5α and BL21(DE3) were used as host strains for recombinant *ospB* constructs in pET9, a T7 polymerase expression vector (encoding kanamycin resistance [55]). The former strain was used as a host during the sequential subcloning stages and was maintained on solid LB medium (39) and propagated in LB broth containing kanamycin (25 μg/ml) where appropriate. The latter strain (F^- *ompT lon*), which contained pLysS (encoding phage T7 lysozyme and chloramphenicol resistance), was used as an expression host for recombinant *ospB* constructs (55). BL21(DE3) with pLysS was maintained on LB solid medium containing chloramphenicol (25 μg/ml) and propagated in LB broth with chloramphenicol. Where appropriate, these media were supplemented with kanamycin (25 μg/ml). All antibiotics were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Antibodies. The MAbs 82C and 84C (both anti-OspB), and an anti-*B. burgdorferi* HB19 WCE polyclonal rabbit serum have been described previously (21). The anti-flagellin MAb H9724, was provided by A. G. Barbour (6). A goat anti-mouse immunoglobulin G (IgG)-horseradish peroxidase (Cappel Research Products, Durham, N.C.) conjugate was used at 1:1,000 in PBSM for Western immunoblotting analyses.

SDS-PAGE and Western immunoblotting. Aliquots of 2.5 μg of borrelial WCE proteins were resolved on discontinuous sodium dodecyl sulfate (SDS)–10% polyacrylamide gels by standard procedures (37). For analysis of recombinant ΔOspB antigens, the trichloroacetic acid-precipitable material from 0.5 ml of *E. coli* culture under induction was dissolved in 25 μl of double-strength Laemmli sample buffer (37), boiled for 5 min, and resolved by SDS–12.5% poly-

TABLE 1. *Borrelia* sp. strains used in this study

Species and strain	Source	Geographic locale	Taxonomic reference	Passage[a]
B. afzelii				
VS461	*Ixodes ricinus*	Switzerland	3, 14, 59	L
FI	*I. ricinus*	Sweden	3, 14	H
J-1 (also known as IPF)	*I. persulcatus*	Japan	3, 41	L
P/Gau	Human skin	Germany	3, 14, 59	L
ACAI	Human skin	Sweden	14	H
ECMI	Human skin	Sweden	3	L
UOI	Human skin	Sweden	3	L
B. burgdorferi sensu stricto				
B31	*I. scapularis*[b]	USA (NY)	1, 3, 14, 45, 59	H
SH-2-82	*I. scapularis*	USA (NY)	41, 45	L
27985	*I. scapularis*	USA (CT)	3, 45, 59	L
CA2-87	*I. pacificus*	USA (CA)	14, 41, 45	H
20004	*I. ricinus*	France	41, 45	L
VS219	*I. ricinus*	Switzerland	14	H
Ip21	*I. persulcatus*	N.W. Russia	3	H
21343	*Peromyscus leucopus*	USA (PA)	3, 45, 59	L
26816	*Microtus* sp.	USA (RI)	3, 45, 59	L
VLI	*Catharus* sp.	USA (CT)	3	L
ECM-NY86	Human skin	USA (NY)	14, 45	L
HB19	Human blood	USA (CT)	45	L
B. garinii				
NBS16	*I. ricinus*	Sweden	14	L
NBS23a	*I. ricinus*	Sweden	14	L
G25	*I. ricinus*	Sweden	3, 14, 45, 59	L
Ip90	*I. persulcatus*	Central Russia	3, 14	H
G1	Human CSF[c]	Germany	41, 45	L
G2	Human CSF	Germany	41, 45	H
Unclassified strains				
DN127 Cl9-2	*I. pacificus*	USA (CA)	59	H
CA-8	*I. pacificus*	USA (CA)		L
CA-25	*I. pacificus*	USA (CA)		L
CA-27	*I. pacificus*	USA (CA)		L
NJ114	*I. scapularis*	USA (NJ)		L
N40	*I. scapularis*	USA (NY)		L
NBS23b	*I. ricinus*	Sweden		L
Lenz	Human skin	USA (NY)		H
MAC13	Human skin	USA (NY)		H
HV-1	Human heart	Austria		H
LV-4 (also known as K59)	Human CSF	Austria		H
Pkal	Human CSF	Germany		L
BB mouse	*P. leucopus*	USA (WI)		L
B. anserina	*Argas persicus*	USA		H
B. hermsii HS-1 (ATCC 35209)	*Ornithodoros hermsi*	USA (WA)		H

[a] H, >10 passages in vitro; L, ≤10 passages in vitro.
[b] *I. scapularis* (also known as *I. dammini*; see reference 44).
[c] CSF, cerebrospinal fluid.

acrylamide gel electrophoresis (PAGE). Gels were run at 20 mA of constant current and cooled by a circulating water heat exchanger. Staining was done with Coomassie brilliant blue R-250 (0.5% [wt/vol]; Sigma) in 35% methanol:10% acetic acid at RT. Molecular weight standards were used in accordance with the supplier's recommendations (BioRad).

Resolved proteins were electrotransferred to PolyScreen polyvinylidene difluoride membranes (New England Nuclear Corp., Boston, Mass.) as described by Towbin et al. (57). Transfer was conducted at 300 mA for 3 h at RT. Posttransfer, membranes were rinsed briefly with PBSM and blocked with PBSM containing 5% (wt/vol) lowfat dry milk for 30 min at RT. Primary screening with MAb supernatants diluted in PBSM plus 0.1% NaN_3 was performed overnight at RT and was followed by three 15-min washes with PBSM at RT. Secondary screening was conducted with goat anti-mouse IgG-horseradish peroxidase (1:1,000 in PBSM) for 1 h at RT. Three subsequent PBSM washes were performed and followed by colorimetric development at RT with imidazole, 4-chloro-1-naphthol, and H_2O_2 as peroxidase substrates (21). Development was terminated by extensive washing with distilled water and air drying of the membrane at RT.

Agglutination assays. The potential surface exposure of the 84C epitope was assessed by testing the ability of MAb 84C (diluted 1:10) to agglutinate borrelias in BSK II. We used an anti-*B. burgdorferi* HB19 WCE polyclonal rabbit serum as a positive control, while MAb H9724 and hybridoma culture medium (Dulbecco modified Eagle medium with 12.5% fetal

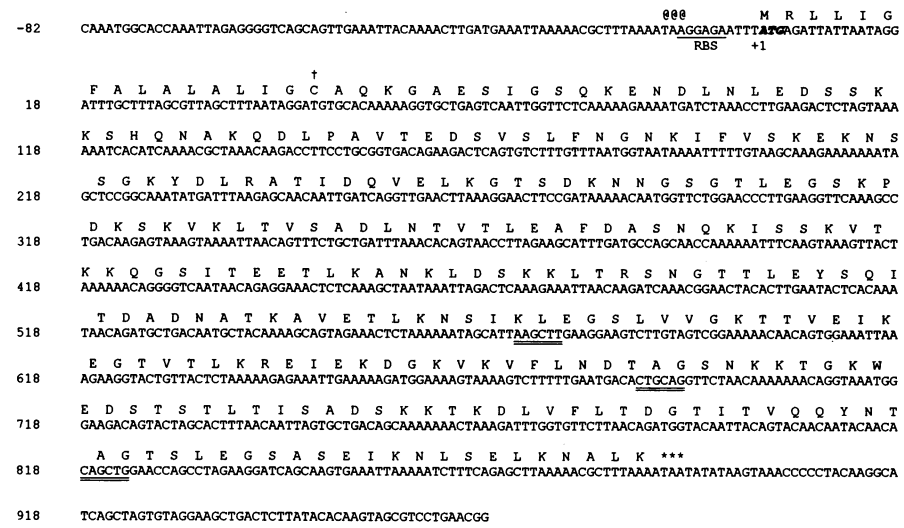

FIG. 1. Nucleotide and amino acid sequence of the *B. burgdorferi* B31 *ospB* gene as reported by Jonsson et al. (36). @@@, stop codon for the upstream *ospA* gene. The proposed ribosomal binding site (RBS) is underlined, and the initiator methionine codon is indicated by boldface-italic text. Nucleotide position 1 corresponds to the first base of the initiator codon. †, putative signal peptidase II processing site for lipoproteins. Three internal restriction endonuclease sites used for deletion mutagenesis are indicated with a double underline. ***, termination codon for the *ospB* gene.

bovine serum, 1.14 mM oxaloacetate, 0.57 mM pyruvate, and 0.2 U of insulin per ml) served as negative controls. MAb H9724 was selected for use as a negative control based on the premise that the periplasmic endoflagella would not be recognized and therefore, only a background level of agglutination would be detected.

Briefly, 180 μl of low passage HB19 mid- to late-exponential-phase borrelias (~10^8 borrelias per ml) were mixed with 20 μl of MAb supernatant, 20 μl of heat-inactivated anti-HB19 rabbit serum, or 20 μl of medium and incubated with no further agitation at 34°C for 6 h. At 6 h, an aliquot was examined by dark-field microscopy for clumping. Five randomly selected fields were examined in a blind fashion by two persons for the number of clumps per field, with the criterion that a group of four or more borrelias with apparent attachment constituted a clump. The data from three experiments were pooled, and the mean number of clumps per field was determined. Student's *t* test was used to compare the mean values for statistical significance.

Selection of 84C antibody escape variants. Antibody-resistant variants of *B. burgdorferi* B31 were isolated under selective pressure from the anti-OspB monoclonal antibody 84C in BSK II plus 6% normal rabbit serum in polystyrene tubes, as described by Šadziene et al. (48). Briefly, heat-inactivated ascitic fluid from an 84C hybridoma ascites tumor was diluted 1:100 in 6 ml of medium containing ~5 × 10^5 borrelias. After incubation at 34°C and detection of growth assessed by phase-contrast microscopy, surviving borrelias were cloned by plate dilution on solid BSK II medium to yield clonal populations of antibody-resistant variants (48).

DNA cloning of *ospB* genes and sequencing analysis. One ml of exponential-phase *Borrelia* sp. culture was harvested by centrifugation and resuspended in 0.1 ml of sterile water. Samples were boiled for 5 min and then cooled to RT on ice. Cellular debris was removed by centrifugation, and 10 μl of the clarified lysate was used for amplification of the *ospB* gene by PCR.

DNA corresponding to a fragment between nucleotide positions −78 and 934 of the *B. burgdorferi* B31 *ospB* sequence (Fig. 1) was amplified by symmetric PCR. Conditions for the PCR were in 50-μl volumes as follows: 10 μl of borrelial lysate; 20 mM Tris HCl (pH 8.75); 10 mM KCl; 10 mM $(NH_4)_2SO_4$; 2 mM $MgCl_2$; 0.1% Triton X-100; 100 μg of bovine serum albumin per ml; 200 μM each dATP, dGTP, dCTP, and dTTP; 50 pmol each of primers B13 and B16 (Table 2); 2.5 U of Stratagene Pfu DNA polymerase (Stratagene, La Jolla, Calif.); samples were overlaid with 40 μl of mineral oil. Amplification cycle conditions consisted of denaturation at 94°C for 30 s, annealing at 50°C for 1 min, and elongation at 72°C for 1 min. Thirty-five amplification cycles were followed by a single elongation cycle at 72°C for 7 min.

Two μl of the PCR-amplification product was cloned into the TA-Cloning Vector system by using an Invitrogen TA Cloning kit as recommended by the vendor (Invitrogen, San Diego, Calif.). Plasmid DNA was prepared by using a Qiagen plasmid Midi kit (Qiagen Inc., Chatsworth, Calif.), and sequenced by elongation from internal primers as previously described (36). Oligonucleotide primers B13 through B16 were used and are depicted in Table 2.

Observed differences between the *ospB* nucleotide sequences of the escape variants and wild-type (WT) B31 were verified by further sequencing of the 3′ region by using a

TABLE 2. Oligonucleotide primers used for PCR and nucleotide sequencing

Oligonucleotide	5′ to 3′ position[a]	5′ to 3′ sequence
B13	−78 to −58	TGGCACCAAATTAGAGGGGT
B14	139 to 158	CAAGACCTTCCTGCGGTGAC
B15	360 to 382	CACAGTAACCTTAGAAGCATTTG
B16	934 to 914	CTTCCTACACTAGCTGATGCC
T7 term	NA[b]	AGGCCCCAAGGGGTTATGC

[a] Nucleotide positions correspond to the *ospB* nucleotide sequence in Fig. 1.

[b] NA, not applicable.

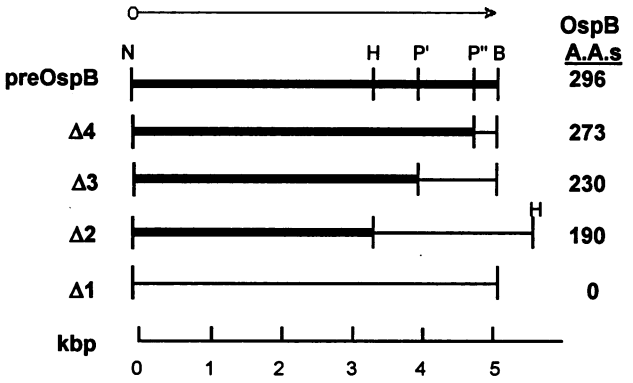

FIG. 2. Restriction endonuclease site map showing the sites utilized in construction of Δ*ospB* mutants for protein analysis of the 84C binding region. The heavier line indicates the region of the *ospB* gene which is present in each of the constructs. Restriction sites are as follows: N, *Nde*I; H, *Hin*dIII; P', *Pst*I; P", *Pvu*II; and B, *Bam*HI.

fragment produced via asymmetric PCR. The asymmetric PCR was performed as described above except for variation in the primer concentrations. To obtain single-stranded DNA templates, 50 pmol of one of the primers in the primer pair set was used along with 0.5 pmol of the other member of the set.

The template for asymmetric PCRs was prepared by separating the products of the symmetric PCR over 0.7% agarose gels in Tris-borate EDTA (39). The desired band was excised, and DNA was extracted by centrifugation as previously described (62). Approximately 10% of the extracted DNA was used as a template for the asymmetric PCR. The product of this asymmetric PCR was purified by using Promega Magic PCR Preps DNA purification system (SDS-Promega, Falkenberg, Sweden). One-third of the gel-purified DNA was used as a template for each sequencing reaction.

The predicted amino acid sequences of each of the two B31 escape variants were compared with the WT sequence by using the PeptideStructure program of the University of Wisconsin Genetics Computer Group Sequence Analysis Software (version 7.3 [22]). In particular, comparisons were made using the Chou-Fasman predictions with respect to secondary structure, antigenic index (34), Kyte-Doolittle hydrophilicity, and surface probability.

Deletion mutagenesis of the *ospB* locus. Similar to what has been described for OspA (24), the entire *ospB* gene of *B. burgdorferi* B31, including the signal sequence and signal peptidase II processing site for lipoproteins, has been cloned into pET9, a T7 RNA polymerase expression vector (25). To facilitate subcloning, the nucleotide sequence of this recombinant *ospB* construct, pET9preOspB, had been altered between the ribosomal binding sequence and the first *ospB* codon to add an *Nde*I restriction endonuclease site and in the 3' untranslated region to add a *Bam*HI site.

A series of four deletions were constructed from the 3' end of the gene by using known restriction endonuclease sites (Fig. 2). Three of these constructions utilized the novel *Bam*HI site at the extreme 3' end of the *ospB*-vector junction while the fourth, pET9preOspBΔ2, involved a *Hin*dIII site 495 bp further downstream (i.e., into the vector) than the *Bam*HI site. Other *ospB* internal sites used included the *Nde*I site at the 5' *ospB*-vector junction for pET9preOspB Δ1, the *Pst*I site at position 690 for pET9preOspBΔ3, and the *Pvu*II site at position 820 for pET9preOspBΔ4. Nucleotide positions were determined from the *ospB* sequence as shown in Fig. 1.

The general scheme for construction of each deletion mutant involved (i) restriction endonuclease digestion, (ii) separation of the digestion product(s) over agarose gels and excision of the band of interest, (iii) elution of the DNA from the gel by agarase digestion, (iv) secondary digestion of the DNA with *Bam*HI, (v) reisolation of the digestion product via agarose gel electrophoresis and elution with agarase, (vi) blunt ending of the noncohesive restriction site overhangs with either T4 DNA polymerase or the Klenow fragment of *E. coli* DNA polymerase, and (vii) blunt-end ligation overnight at RT. *E. coli* DH5α was transformed with the religated constructs by the method of Cohen et al. with selection on LB solid media containing kanamycin (18).

Specifically, the digestions leading to pET9preOspBΔ1 (*Bam*HI and *Nde*I), pET9preOspBΔ2 (*Hin*dIII), and pET9 preOspBΔ4 (*Bam*HI and *Pvu*II) were blunt ended with the Klenow fragment of *E. coli* DNA polymerase, and the *Bam*HI-*Pst*I digests for pET9preOspBΔ3 were blunt ended with T4 DNA polymerase (39). All restriction enzymes and DNA modification enzymes were purchased from Promega and used in accordance with the supplier's recommendations (Promega Corp., Madison, Wis.). Agarase was used in accordance with the supplier's recommendations (Boehringer Mannheim Biochemicals, Indianapolis, Ind.).

Isolated transformant colonies were selected and passaged once on solid LB medium plus kanamycin to confirm the kanamycin-resistant phenotype. Kanamycin-resistant clones were amplified in 2.5 ml of LB broth plus kanamycin (25 μg/ml) and used to isolate plasmid DNA by an alkaline lysis miniprep method (39). Recovered plasmid DNA was screened by restriction endonuclease digestion to verify the loss of specific sites due to the deletion process. Recombinant Δ*ospB* clones having the predicted restriction site patterns were used to transform the expression strain, *E. coli* BL21(DE3) containing pLysS with selection of transformants on LB solid medium plus chloramphenicol and kanamycin (both at 25 μg/ml). Isolated transformants were passaged a second time on selective media to verify the stability of the kanamycin-resistant phenotype.

To identify and characterize the 3' junctions of the recombinant *ospB* deletion constructs, DNA sequencing was performed at the Center for Advanced DNA Technologies (University of Texas Health Science Center, San Antonio). Briefly, the methodology involved cyclic sequencing of double-stranded DNA templates by chain termination using a thermal cycler. The labeling reactions involved the *Taq* DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, Calif.) with fluorescent dye labeling of elongated templates via AmpliTaq DNA polymerase activity, as recommended by the vendor (Applied Biosystems Inc.). DNA templates were prepared by an alkaline lysis miniprep procedure with two phenol:chloroform and two chloroform extractions followed by two ethanol precipitations. For each reaction, 1 μg of template DNA and 3.2 pmol of primer were mixed prior to addition of deoxynucleoside triphosphates (dNTPs) to 7.5 μM, the DyeDeoxy terminator dideoxy NTPs, and 0.2 U of AmpliTaq polymerase. Denaturation was at 96°C for 15 s followed by 1 s of annealment at 50°C and 4 min of extension at 60°C per cycle, for 25 cycles. Sequencing products were separated and analyzed by using the Applied Biosystems Model 373A DNA sequencing system.

The T7 term and B15 primers used in this sequencing phase are depicted in Table 2. The T7 term primer is

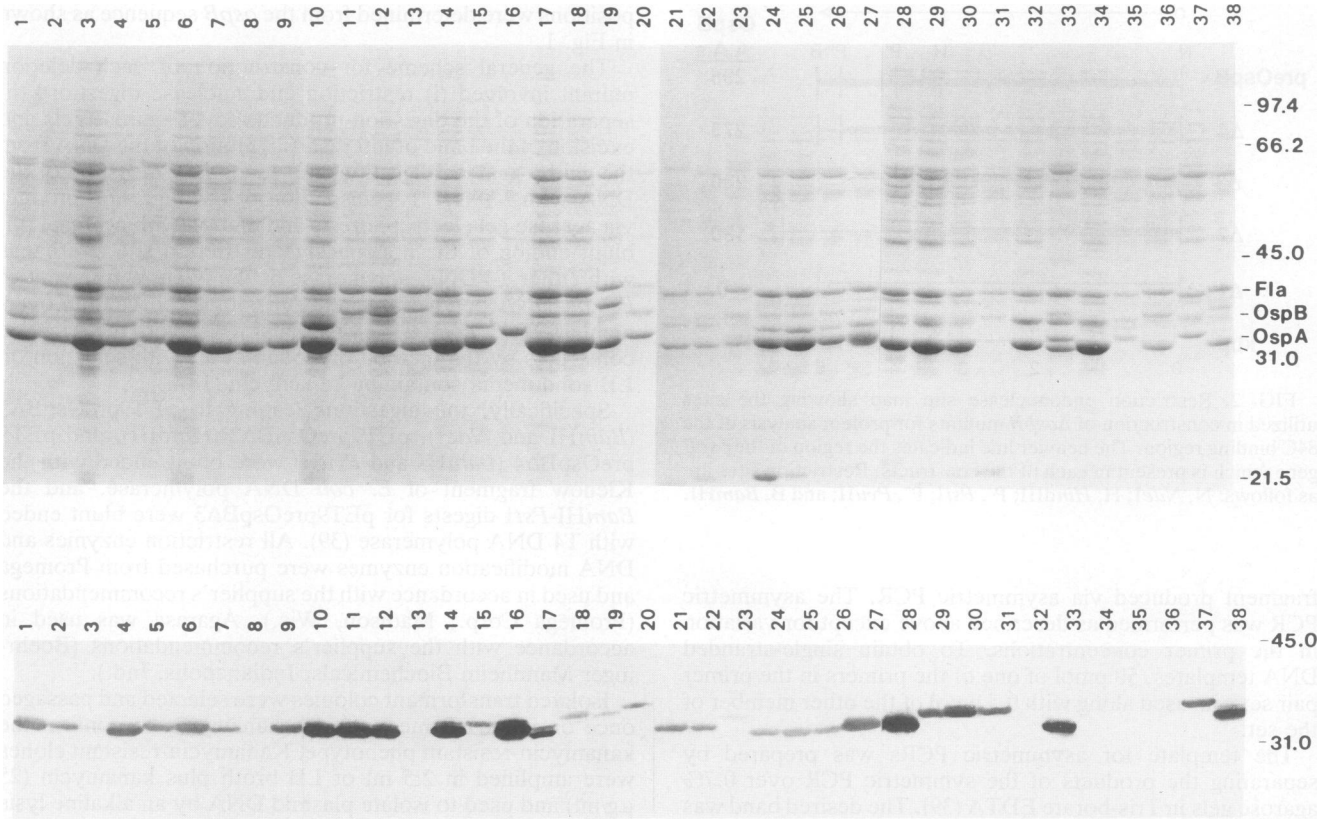

FIG. 3. Washed-cell extracts of *Borrelia* spp. analyzed by SDS–10% PAGE with Coomassie blue staining (upper panel) and Western immunoblot analysis with MAb 84C (lower panel). Positions of the molecular weight standards are indicated on the right margin (BioRad). Each lane contains 2.5 μg of protein arranged primarily by geographic source as follows: 1, B31; 2, Lenz; 3, ECM-NY86; 4, MAC13; 5, 26816; 6, BB mouse; 7, 21343; 8, VLI; 9, SH-2-82; 10, NJ114; 11, 27985; 12, N40; 13, DN127 Cl9-2; 14, CA-2-87; 15, CA-8; 16, CA-25; 17, CA-27; 18, Ip21; 19, Ip90; 20, J-1 (also known as IPF); 21, 20004; 22, VS219; 23, VS461; 24, HV-1; 25, LV-4 (also known as K59); 26, G1; 27, G2; 28, Pka1; 29, P/Gau; 30, ACAI; 31 ECMI; 32, FI; 33, G-25; 34, NBS16; 35, NBS23a; 36, NBS23b; 37, UOI; and 38, HB19.

homologous to the antisense strand of the phage T7 Tϕ gene transcriptional terminator (55) and allowed for sequencing across the Δ*ospB*::pET9 junction in all constructs except for pET9preOspBΔ2. The B15 primer allowed for sequencing of the junction from within the Δ*ospB* gene in constructs other than pET9preOspBΔ1.

Induction and expression of recombinant OspB. Recombinant Δ*ospB* clones in BL21(DE3), also containing pLysS, were incubated overnight with rotation at 37°C in 25 ml of LB broth with chloramphenicol and kanamycin (both at 25 μg/ml). Each culture was diluted in 50 ml of fresh LB broth plus antibiotics to an optical density at 600 nm of ~0.1 and incubated at 37°C and 200 rpm to an optical density at 600 nm of 0.6 to 1.0 (i.e., midexponential phase), upon which time, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 0.5 mM and an additional 25 μg of kanamycin per ml was added. Incubation was continued at 37°C with vigorous aeration for 2 h with 5-ml samples removed at 0, 5, 15, 30, 60, and 120 min postaddition of IPTG. Macromolecules in these 5-ml samples were precipitated by the addition of 5 ml of cold 10% (wt/vol) trichloroacetic acid and incubation for >30 min on ice. Precipitates were pelleted at 12,000 × *g* for 30 min at 4°C, washed twice with 1 ml of 5% trichloroacetic acid, and washed twice more with 1 ml of ethanol:petroleum ether (1:1 [vol/vol]). The solvents were evaporated overnight at 37°C and proteins were solubilized by boiling for 5 min in 0.25 ml of double-strength Laemmli sample buffer (37). After boiling, insoluble debris was removed from suspension by centrifugation at 12,000 × *g* for 5 min and 25 μl of sample (i.e., 0.5 ml of culture) was analyzed by SDS-PAGE and Western immunoblotting (see previous section).

RESULTS

Strain reactivity with 84C. A total of 38 strains of *B. afzelii*, *B. burgdorferi*, and *B. garinii* were tested for the presence of an 84C-reactive antigen by Western immunoblotting (Fig. 3). Thirty-two (84.2%) of the strains tested reacted with the MAb, with both low- and high-passage isolates showing reactivity. Strains which tested positive for 84C-reactivity included isolates from *Ixodes* sp. ticks, humans, and other mammals. Reactive isolates were observed to come from North America, Europe, Russia, and Japan, suggesting that MAb 84C recognized an extremely highly conserved epitope of OspB. The exceptions included two North American strains, VLI and DN127 Cl9-2, and four Swedish isolates, FI, NBS16, NBS23a, and NBS23b. Conservation of the 84C epitope was also observed to be restricted to the Lyme disease borrelias, as two other *Borrelia* spp. tested, *B. anserina* and *B. hermsii*, were both 84C nonreactive, as were three different virulent *Leptospira interrogans* serotypes: canicola, copenhageni, and icterohaemorrhagiae (data not shown).

Under current classification schemes, VLI is a *B. burg-*

dorferi sensu stricto strain (3), NBS16 and NBS23a are *B. garinii* strains (14), FI is a *B. afzelii* strain (3, 14), and DN127 Cl9-2 (59) and NBS23b have not been identified to the species level at this time. Of these six 84C-nonreactive strains, only DN127 Cl9-2 reacted with the anti-OspB carboxyl terminus MAb 82C (data not shown). When the protein profiles of these 84C-nonreactive strains were examined, FI and NBS16 appeared to be expressing an abundant protein of ~34 kDa which may be an OspB (Fig. 3A, lanes 32 and 34), while the other four strains appear to lack an OspB protein in this size range (Fig. 3A, lanes 8, 13, 35, and 36).

The VLI isolate is from a veery (*Catharus* sp.; 2), a small species of songbird, and has been reported to lack an apparent OspB protein of 34 kDa by Coomassie blue staining analysis (7). The DN127 Cl9-2 strain, a cloned variant of the original DN127 isolate, was recovered from *Ixodes pacificus* and was reported to lack OspA and OspB by Western blot and Coomassie blue staining analyses (13). The four Swedish isolates are all *I. ricinus* isolates with the three different NBS strains recovered from two ticks captured on Norrbyskär, an island in the Gulf of Bothnia (12). The three NBS strains are considered to be different isolates by a variety of criteria (12) and substantiated by our Coomassie blue staining analysis (Fig. 3A, lanes 34 to 36).

Deletion mutagenesis of recombinant OspB. A set of four carboxy-terminal deletion mutants was constructed from the full-length pET9preOspB clone (Fig. 2). Western immunoblot analysis of induced Δ*ospB* gene products demonstrated that the epitope recognized by 84C was contained within amino acids 230 and 273 (Fig. 4A). The amino acid residue numbers are taken from the predicted amino acid sequence of the B31 *ospB* (Fig. 1). Figure 4B demonstrates that three of the four Δ*ospB* constructs produced a truncated OspB that reacted with the anti-OspB MAb 82C, previously determined to react with an amino-terminal epitope in OspB (21). As predicted, the exception to this observation was pET9preOspBΔ1, which was deleted for the entire *ospB* coding sequence (Fig. 4B, lane 3).

The observed molecular sizes of the largest band for each Δ*ospB* construct agreed closely with those predicted from DNA sequence analysis, given in parentheses as follows: pET9preOspB, 34.0 kDa (35.2 kDa); pET9preOspBΔ2, 22.0 kDa (22.6 kDa); pET9preOspBΔ3, 26.5 kDa (28.1 kDa); and pET9preOspBΔ4, 32.5 kDa (32.9 kDa). DNA sequencing analysis indicated that pET9preOspBΔ2 contained an additional 3 amino acids (AYR) as a product of the religated blunt ends. Similarly, pET9preOspBΔ3 contained an additional 9 amino acids (IRLLTMPGR) and pET9preOspBΔ4 contained an additional 4 amino acids (GSGC) (data not shown).

The presence of a band at ca. 34 kDa in the pET9preOsp BΔ4 lanes (lane 6) is suggestive of the precursor form of the *ospB* gene product (i.e., preOspBΔ4 with an intact signal sequence) which would have been recovered in a trichloroacetic acid precipitate of this culture. The presence of several reactive bands smaller than the predicted molecular size is suggestive of degradation of the truncated OspB molecules. The observation that these bands were reactive with 82C and not with 84C suggests that the putative degradation was occurring from the carboxy-terminal end of the protein.

Agglutination assays. MAb 84C was observed to yield significantly more clumped borrelias per field than treatment with hybridoma medium or the periplasmically directed MAb H9724 (all diluted 1:10). When borrelias were incubated with 84C, a mean of 4.6 ± 3.1 clumps was observed per field, while incubation with medium or H9724 produced

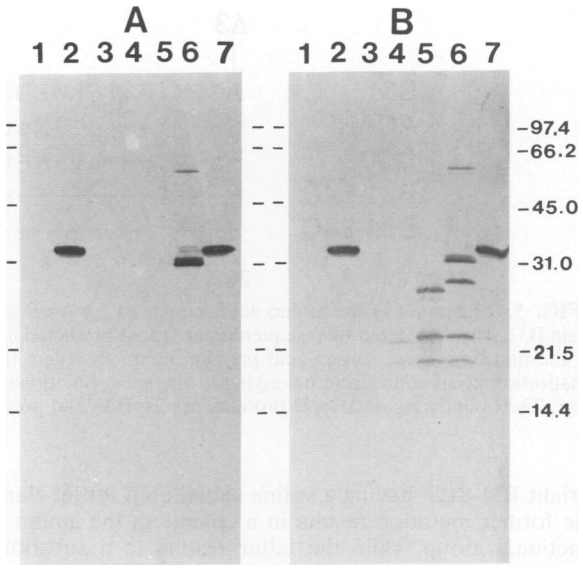

FIG. 4. Western immunoblot analyses of T7 RNA polymerase-induced proteins in *E. coli* BL21(DE3) probed with anti-OspB monoclonal antibodies. Trichloroacetic acid-precipitable materials from 0.5 ml of culture was loaded per lane of an SDS–12.5% polyacrylamide gel. Lanes contain material from BL21(DE3) containing pLysS as well as the following constructs: 1, no second plasmid; 2, pET9preOspB; 3, pET9preOspBΔ1; 4, pET9preOspBΔ2; 5, pET9preOspBΔ3; 6, pET9preOspBΔ4; and 7, pET9preOspB. Panel A was probed with MAb 84C, and panel B was probed with MAb 82C. Positions of molecular weight standards are indicated on the right margin (BioRad).

1.9 ± 1.2 and 1.5 ± 1.2 clumps per field, respectively ($P < 0.05$). These data suggested that the epitope recognized by 84C was expressed on the surface of the borrelias.

Isolation of 84C escape variants of *B. burgdorferi* B31. Following growth of B31 borrelias in liquid BSK II containing the MAb 84C, two independent surviving variants were observed by phase-contrast microscopy. These variants were cloned by limiting plate dilution and expanded for characterization. Western immunoblot analysis with MAb 84C demonstrated that variant B31-84C failed to react with the selecting MAb yet produced an OspB of 34 kDa which reacted with other anti-OspB MAbs, while the second variant, B31-84C′, still reacted with MAb 84C in Western immunoblot analysis. According to the classification scheme defined by Šadziene et al., B31-84C would be considered a class III mutant and B31-84C′ would be considered a class IV mutant (48). The ability to isolate 84C escape variants is also strongly suggestive of surface exposure for the 84C epitope.

DNA sequencing analysis of WT and escape mutant *ospB* alleles. The nucleotide sequences of the *ospB* loci from three strains of Lyme disease borrelias have been reported previously (36). The nucleotide sequence of the mutant *ospB* alleles of B31-84C and B31-84C′ were each observed to differ at a single nucleotide. Variant B31-84C′ had a C-to-T transversion at nucleotide 749, while variant B31-84C had an A-to-G transition at position 788 (data not shown). When these altered bases were aligned with the sequence of the WT *ospB* alleles, each resulted in a single amino acid change (Fig. 5). Both of these amino acid changes were observed to occur within a 14-amino-acid long region, with variant B31-84C having a glycine substituted for an aspartic acid and

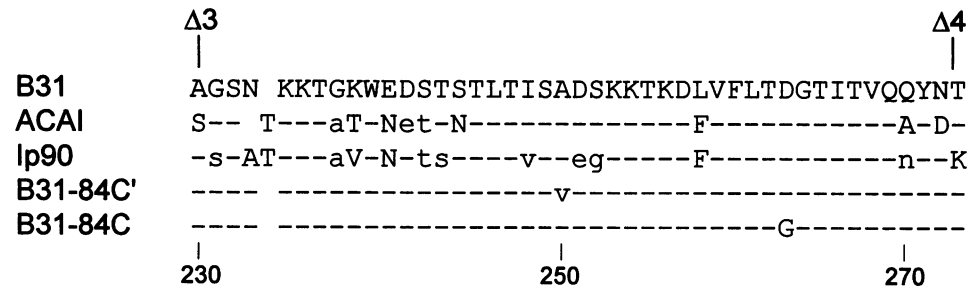

FIG. 5. Alignment of the amino acid sequences for *ospB* genes from three strains of *Borrelia* spp. and two different escape variants of strain B31. The ACAI and Ip90 sequences are those predicted by Jonsson et al. (36) and the sequences of B31-84C' and B31-84C are presented for the first time here. Amino acid position numbers reflect the sequence of *B. burgdorferi* B31 (as in Fig. 1). Lowercase letters indicate substitution of an amino acid belonging to the same functional class of amino acid, while uppercase letters indicate a change in functional class. The boundaries of ΔOspB proteins preOspBΔ3 and preOspBΔ4 are indicated along the top of the figure.

variant B31-84C' having a valine substituted for an alanine. The former mutation results in a change in the amino acid functional group while the latter results in a substitution within the same functional class of amino acids.

Since the mutation in B31-84C resulted in a change in reactivity on Western immunoblot analysis, this datum strongly suggested that the 84C epitope involved residue 263. Also, the alteration in B31-84C' which apparently prevented antibody recognition in situ, based on the ability of this variant to "escape" antibody selection with 84C while the *ospB* gene product was still 84C reactive on Western immunoblots, suggests that the change from A to V at residue 250 alters the lipoprotein's conformation and thereby converts the 84C epitope from a surface exposed locale (in the WT) to a buried, antibody-inaccessible locale in the mutant.

Comparing the predicted amino acid sequences of the variants with the WT B31 sequence suggested that a difference in the predicted Chou-Fasman secondary structure of B31-84C' occurs at position 250 (the site of the A-to-V substitution), resulting in the loss of a turn in the sequence (Fig. 6). The predicted secondary structures of the WT and B31-84C were observed to be identical over their entirety (Fig. 6).

Similar analyses were performed to examine various predicted parameters of the variants which suggested some minor differences. An additional hydrophobic region was observed in B31-84C as compared with B31-84C' and the WT (Kyte-Doolittle analyses with a threshold level of ≥1.3; data not shown), likely the D-to-G substitution at amino acid 263. This prediction of increased hydrophobic character also substantiated the effect of the D-to-G substitution in OspB-84C. Secondly, the region just carboxy terminal from amino acid 250 of B31-84C' was predicted to have a slightly lower antigenic index than either B31-84C or the WT (threshold level of ≥1.2; data not shown). These data offer support for the above stated hypothesis regarding the observed difference in the 84C reactivities of OspB-84C' in its denatured versus in situ forms. Lastly, no predicted differences were suggested when surface probabilities were compared (threshold level of ≥5.0; data not shown). These data would seem to contradict the hypothesis regarding conformational alteration due to the B31-84C' mutation. However, since all of these predictions are based on a two-dimensional secondary structure, this may not accurately reflect the conformation of OspB in situ.

When the amino acid sequences of three divergent species of Lyme disease borrelias were compared, a significant region of identity was observed between residues 259 and 269 (position numbers relative to B31 sequence; Fig. 5). On the basis of these data, we propose that the 84C epitope resides between amino acids 259 and 269.

DISCUSSION

The outer surface lipoprotein B (OspB) has been characterized from many strains of the *Borrelia* spp. which cause Lyme disease in humans. Two of the observed hallmarks of this lipoprotein have been (i) a high degree of antigenic heterogeneity and an apparent lack of genetic stability in laboratory media (8, 9, 16, 30, 46, 61) and (ii) an apparent dispensability for growth in laboratory media (16, 19, 40, 43, 47, 48, 51). We present data here describing an anti-OspB MAb, 84C, which demonstrated a high degree of cross-reactivity among many strains of diverse animal and geographic origins. We were able to delimit the region of OspB recognized by this MAb to a 11-amino-acid highly conserved, surface-exposed region in the carboxyl terminus of the OspB molecule through the use of Δ*ospB* recombinant antigens and specific 84C-resistant escape variants of *B. burgdorferi* B31.

While OspB$^-$ borrelias are only rarely found among patient isolates (61), there have been a small number of reports describing Lyme disease borrelia isolates, mainly from ticks, which lacked apparent expression of an OspB (2, 12, 13, 26). The *Ixodes pacificus* isolate DN127-Cl9-2 was reported to lack OspB as assessed by Coomassie blue staining (13) and confirmed in the present study (Fig. 3A, lane 13) and by Western immunoblot analysis with two other anti-OspB MAbs, H6831 and H5TS (13). In the present study we observed that while DN127 Cl9-2 failed to react with MAb 84C (Fig. 3), the strain did express a small amount of a ~34-kDa antigen that was recognized by the anti-OspB amino-terminal MAb 82C (data not shown). The data suggest the production of a rapidly degraded OspB or an *ospB* locus lacking the conserved 84C block near the 3' terminus. Three *I. ricinus* isolates from northern Sweden, NBS16, NBS23a, and NBS23b (12), the latter two of which appeared to lack an OspB by Coomassie blue staining analysis, all failed to react with either of the two anti-OspB MAbs tested here, suggesting the complete lack of, or extremely low expression of, an *ospB* gene product. The German *I. ricinus* isolate GÖ2 reportedly possesses an intact *ospAB* operon, but the *ospB* locus contains several stop codons in frame with the putative ribosomal binding site (26). The 84C reactivity of GÖ2 was not tested in this study. VLI, a North American bird isolate,

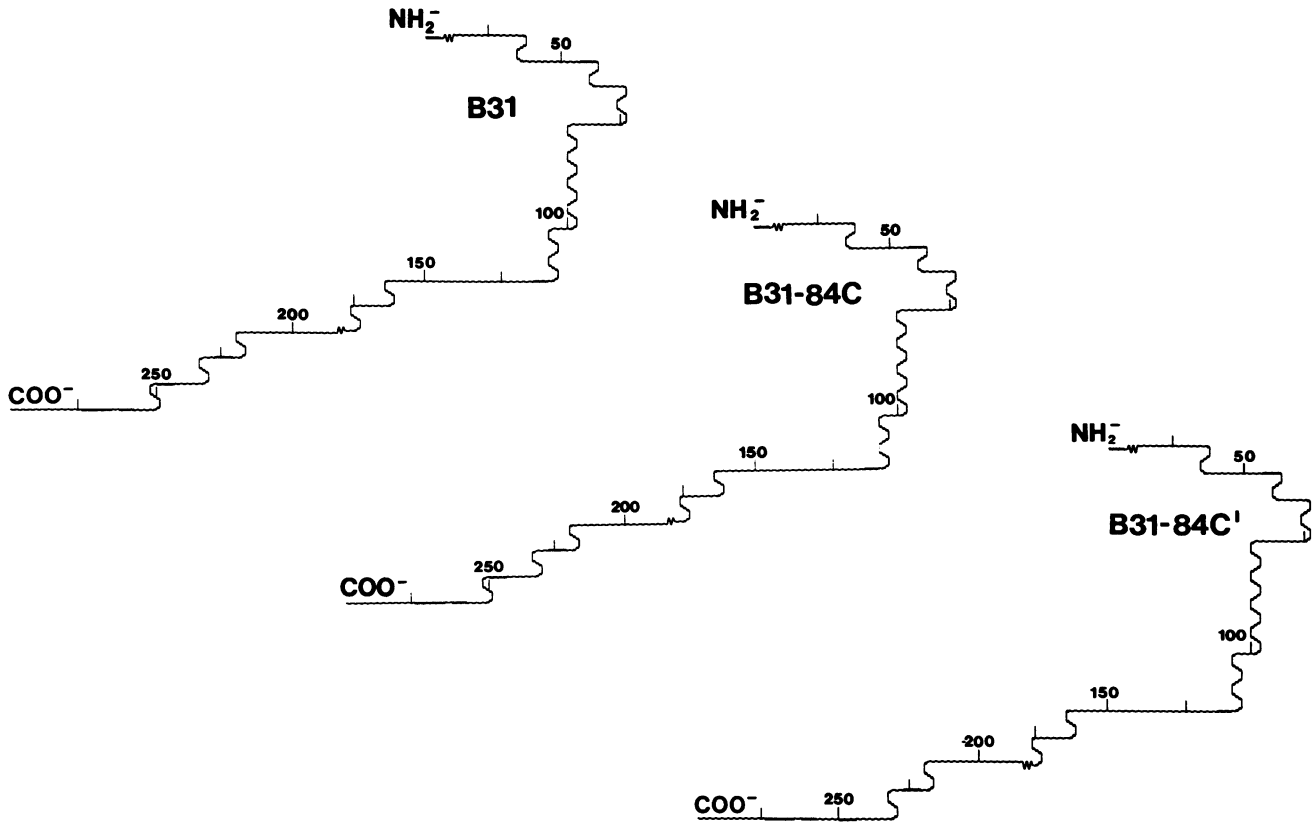

FIG. 6. Two-dimensional depiction of the predicted secondary structures of the carboxy-terminal region of OspB from B31, variant B31-84C, and variant B31-84C'. The sequences were compared by using the PeptideStructure program of the GCG sequence analysis package (22) with Chou-Fasman predictions of Kyte-Doolittle hydrophilicity and hydrophobicity. Predicted turns are depicted by 180° changes in direction. The amino and carboxy termini are indicated, and amino acid position numbers are given for reference on each plot.

was also nonreactive with both 84C and 82C on Western blots in this study and did not appear to express an OspB by Coomassie blue staining analysis (Fig. 3 and data not shown) (7). To our knowledge, VLI and DN127 Cl9-2 are the only ones of these reported OspB-deficient isolates to have been tested for their virulence capacities. VLI was reported as showing infectivity in chick embryo and hamster model systems (2), while DN127 Cl9-2 was reported to be avirulent in the LEW/N rat model system (10). However, the DN127 Cl9-2 was tested at a passage number of >20, which may have been the cause of the observed avirulence (10).

In the more recently described mouse model systems, expression of an *ospB* gene product has been correlated with virulence (47, 52). However, recently, two reports have been issued regarding the importance of the carboxyl terminus of OspB in virulence. The first report describes a naturally occurring variant of *B. burgdorferi* N40 which has a stop codon at amino acid position 192 (Fig. 1), producing a truncated OspB. While the infectivity of the mutant was not examined quantitatively, this variant was able to persist in C3H/HeJ mice immunized with a heterologous, full-length OspB, causing arthritis and endocarditis (30). Passive protection experiments with a polyclonal antiserum directed against a homologous, full-length OspB demonstrated complete protection in a challenge with the truncated OspB variant, while the anti-OspB carboxyl terminus-directed MAb, 7E6C, failed to confer protection against this truncated OspB variant (30).

In a second study, an anti-OspB escape variant of *B. burgdorferi* SH-2-82, selected with MAb H614, was shown to be 1.7 to 2.3 orders of magnitude less infective than the WT strain in CB-17 *scid* mice (47). However, this study did not examine the ability of the escape variant to cause disease. The escape variant, which terminates its OspB at amino acid 277 (number as in Fig. 1), was also reported to penetrate human endothelial cell monolayers only 37% as well as its WT parent strain, supporting the reduced virulence observed in vivo (47).

This SH-2-82 escape variant, selected with H614, also failed to react with MAb 84C by Western blot analysis (47), identifying it as a class II mutant by the scheme of Šadziene et al. (48). Since the actual point of translational termination was carboxy terminal to the 84C epitope proposed in this study, the lack of reactivity with 84C may be due to the alteration of amino acids 270 through 277 (QYNTAGTS in the WT and HTTQLEPA in the mutant) (47). More likely, though, the failure to react with 84C is due to degradation of the truncated protein, as proposed by Šadziene et al., as the authors were unable to detect any OspB-like proteins larger than 18 kDa by Western immunoblot analyses (47).

A third study implicates a potential role for OspB in the in vitro association of *B. burgdorferi* HB19 with human endothelial cells. Thomas and Comstock reported that three different anti-OspB MAbs (FR14, FR8, and H5TS) each showed some level of inhibition of the ability of borrelias to associate with cultured human endothelial cells (56). How-

ever, since this study did not differentiate attachment from invasion, these data can not be compared equivalently with that in the recent report by Šadziene et al. (47). Also, this earlier study (56) utilized intact IgG molecules rather than isolated Fab fragments, which may have resulted in an artifactual inhibitory effect, as a later study by our laboratory examined the ability of 8 different anti-OspB Fab preparations (84C inclusive) to inhibit borrelial-endothelial cell association and observed no inhibitory effects (21). Nonetheless, these studies do suggest a potentially functional role for OspB in the pathogenesis of Lyme disease.

We localized the region of OspB recognized by MAb 84C to a highly conserved 11-amino-acid region near the carboxy-terminal end of the protein (Fig. 4 and 5). This was accomplished via two lines of evidence. First, Western immunoblot analysis of a set of 3′-deleted recombinant OspB antigens demonstrated that the 84C binding site was within a 43-amino-acid region, bounded by residues 230 and 273 in strain B31 (Fig. 4). Secondly, DNA sequence analysis of two independently arising strain B31-84C escape variants demonstrated a single point mutation in each variant. These two variants each possessed one altered amino acid clustered within a 14-amino-acid-long region (Fig. 5). However, since B31-84C′ was still Western immunoblot reactive with MAb 84C, the role of the A-to-V change in this region is thought to be unimportant in the context of MAb binding. This hypothesis was supported by computer analyses of the amino acid sequences of the variants and the WT OspBs. The substitution at position 250 results in a predicted change in secondary structure (Fig. 6) which could affect reactivity in situ, but not in denatured form if the epitope was linear.

The 84C epitope is presumed to be linear in nature on the basis of (i) the reactivity with the MAb observed after Western blotting (Fig. 3 and 4); (ii) the observations that the MAb reacted with borrelias which had been fixed to glass slides with acetone, glutaraldehyde, heat, or methanol (data not shown); and (iii) the predicted secondary structures, antigenic indices, and surface probabilities of the 84C variant and the WT which were identical (Fig. 6 and data not shown).

Considered together, these data suggest that the most likely location for the 84C epitope is between amino acid residues 259 and 269 of the B31 sequence (Fig. 5). Interestingly, the predicted amino acid sequence for the *ospAB* operon from a European tick isolate, *B. burgdorferi* sensu lato strain B29, has been reported and shows 100% identity over this range as well (27).

The passage number of some of the strains appeared to affect the expression of the 84C epitope. In a previous study, when *B. burgdorferi* HB19 was assessed by Western immunoblot, low-passage borrelias were 84C reactive while high-passage borrelias (i.e., >50 passages from initial isolation) failed to react with 84C (53). However, in this study, when many strains passaged more than 11 times were analyzed (n = 13), 11 of these reacted with 84C, so there is not an absolute relationship between passage number and expression of the OspB epitope (Table 1 and Fig. 3).

Surface exposure of the epitope was initially reported by Comstock et al. (21) and confirmed here by aggregation experiments utilizing a polyclonal anti-*B. burgdorferi* HB19 antiserum as a positive control and the anti-flagellin MAb H9724, as well as hybridoma culture medium, as negative controls. Additional evidence for surface exposure of the 84C epitope is demonstrated by the ability to isolate antibody-resistant escape variants of a strain grown in the presence of the MAb. Previous studies have demonstrated that MAbs directed against non-surface-exposed epitopes (e.g., H9724) are extremely inefficient and do not allow for the selection of escape mutants (46a).

Apparent degradation of the recombinant ΔOspB molecules induced in *E. coli* BL21(DE3) was detected by Western immunoblot analysis (Fig. 4B), even though this host strain lacks the Lon and OmpT proteases (55). This degradation may be due to a destabilized tertiary structure for the truncated proteins which allows for proteolytic degradation of these "foreign proteins" by the host cell (32), as the full-length OspB showed comparatively less degradation (Fig. 4B). Also, the 15 min post-induction time point used for the stained gel and Western blot shown in Fig. 4 was selected because it showed less degradation of the ΔOspB proteins than did longer time points (up to 120 min; data not shown). This unstable conformation of the ΔOspBs could be a result of either (i) the missing OspB amino acids or (ii) the presence of novel amino acids added as a result of the blunt-end ligation procedure. It is unknown at the present time which of these two possibilities is responsible for this apparent instability or whether some other mechanism is responsible.

There have been reports evaluating the efficacy of OspB-based vaccines in laboratory mice on the prevention of Lyme disease, which have shown some level of protection against heterologous strains (29, 30). The protective epitopes of OspB were also mapped to the carboxy-terminal half of OspB by the use of fusion peptides and a naturally occurring ΔOspB-expressing variant (30). These data, together with that of Šadziene et al. (47), suggest that the carboxy terminus of OspB plays a role in pathogenesis of Lyme disease. However, the former study also described a naturally occurring mutant of strain N40, expressing a truncated OspB, that was demonstrated to be virulent in C3H/HeJ mice (30). Therefore, at this time, the role of OspB in pathogenesis is still not definitively understood.

The data presented here, notably the demonstration of a highly conserved domain of OspB, in a region of the lipoprotein which has been proposed to function in virulence, suggest that this domain may play an important functional role in some aspect of the bacterium's existence. This potential function, once defined, would explain the conservation of the 84C domain among diverse strains from diverse regions. While OspB-less strains have been isolated, mainly from ticks, there are few reports describing the virulence of any of these isolates in accepted animal model systems.

It is not an entirely farfetched idea that perhaps mixed populations of *Borrelia* spp. with differing virulence capabilities may exist in *Ixodes* sp. ticks. While most of these strains can encode for an OspA-like antigen which is reactive with H5332 (commonly used in indirect immunofluorescent analyses along with the genus-specific H9724 MAb to identify spirochetes as Lyme disease borrelias), what may differentiate these mixed virulent and avirulent populations of borrelias could be the expression of other virulence factors (e.g., OspB). More careful analysis of the data regarding isolation of "Lyme disease borrelias" from animal sources, in particular from ticks, may help to explain this hypothesis.

Another important aspect of Lyme disease with regard to the 84C domain may be the use of this conserved antigen in diagnostic assays. Some of the major problems associated with the effective laboratory diagnosis of Lyme disease are (i) failure to recover sufficient organisms for culture or antigenic analysis from patient specimens, (ii) the observed time lag in the development of host antibody responses to the well-characterized borrelial antigens, (iii) the existence of cross-reactive antigens among Lyme disease borrelias and

other pathogenic spirochetes (38), and (iv) the lack of a good marker antigen for Lyme disease spirochetes that is highly conserved, yet restricted to these species. We propose further study of the 84C domain to examine the potential value of using this MAb as a tool for an assay based on antigen capture. One example of such an assay has been described by Dorward et al. (23). Since OspB is immunogenic in patient sera, we presume that it is expressed in vivo and that some amount of OspB antigen would be shed into the blood and/or the urine and could therefore be detected in these samples. Dorward et al. reported the ability to detect borrelial antigens in the urine of infected dogs and mice and in the urine of diagnosed Lyme disease human patients (23). The antigen capture system that these investigators used reacted with OspA and OspB on Western immunoblots, and reportedly was able to detect borrelial antigens by immunoelectron microscopy in the urine of infected mice at dilutions of up to 10^6, indicating a heavy antigen load in the urine (23). Because of the highly conserved nature of the 84C antibody-reactive domain among patient isolates (13 of 13 tested) and the antigenic specificity of MAb 84C for Lyme disease borrelias, we feel that the 84C domain may be a relevant test antigen for use in diagnosis.

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